Amendments to the Specification

In the Specification:

Please insert the following paragraph on page 1 after the Title:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a national phase of International Appl. No. PCT/US2004/011895, filed April 16, 2004, which published under PCT Article 21(2) in English, said PCT/US2004/011895 claims the benefit of U.S. Provisional Appl. No. 60/463,724, filed April 18, 2003, both of which are herein incorporated by reference.

Please replace paragraph [0005] with the following amended paragraph:

[0005] MHC molecules are highly polymorphic proteins that regulate T cell responses (Schwartz, B. D., *The human major histocompatibility complex HLA in basic & clinical immunology* Stites *et al.*, eds., Lange Medical Publication: Los Altos, pp. 52-64, 4th ed.). The species-specific MHC homologues that display CTL epitopes in humans are termed human leukocyte antigen ("HLA"). HLA class I molecules can be divided into several families or "supertypes" based upon their ability to bind similar repertoires of peptides. Vaccines which bind to multiple HLA supertypes, such as for example A2, A3, and B7, will afford broad, non-ethnically biased population coverage. As seen in Table **9** **[[1]]**, population coverage is approximately 84-90% for various ethnicities, with an average coverage of the sample ethnicities at approximately 87%.

Please replace paragraph [0027] with the following amended paragraph:

[0027] The peptides and corresponding nucleic acids and compositions of the present invention are useful for stimulating an immune response to TAAs by stimulating the production of CTL and optionally HTL responses, *e.g.* therapeutic prophylaxis, and are also useful for monitoring an immune response, *e.g.*, diagnosis and prognosis. The peptides, which contain A2 epitopes derived directly or indirectly (*i.e.* by analoging) from native TAA protein amino acid sequences, are able to bind to HLA molecules and stimulate an immune response to TAAs. The complete sequence of the TAAs proteins analyzed described as SEQ ID NOs:11-15 herein can be obtained from GenBank. See Table **11** **[[2]]**.

Please replace paragraph [0028] with the following amended paragraph:

[0028] The epitopes of the invention have been identified in a number of ways, as will be discussed below. Also discussed in greater detail is an embodiment of the invention in which analogs have been derived wherein the binding activity for HLA molecules or T cell receptor molecules was modulated by modifying specific amino acid residues to create analogs which exhibit altered (*e.g.*, improved) immunogenicity.**[[.]]**

Please replace paragraph [0039] with the following amended paragraph:

[0039] An "epitope" is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site recognized by an immunoglobulin, T cell receptor or HLA molecule. Alternatively, an epitope can be defined as a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by

T cell receptor proteins and/or Major Histocompatibility Complex (MHC) receptors. Epitopes are present in nature, and can be isolated, purified or otherwise prepared or derived by humans. For example, epitopes can be prepared by isolation from a natural source, or they can be synthesized in accordance with standard protocols in the art. Synthetic epitopes can comprise artificial amino acid residues, "amino acid mimetics," such as D isomers of naturally-occurring L amino acid residues or non-naturally-occuring amino acid residues such as cyclohexylalanine. Throughout this disclosure, epitopes may be referred to in some cases as peptides or peptide epitopes. The epitopes and analogs of the invention are set forth in Table **1** **[[3]]**.

Please replace paragraph [0044] with the following amended paragraph:

[0044] An "HLA supertype or HLA family", as used herein, describes sets of HLA molecules grouped on the basis of shared peptide-binding specificities. HLA class I molecules that share somewhat similar binding affinity for peptides bearing certain amino acid motifs are grouped into such HLA supertypes. The terms HLA superfamily, HLA supertype family, HLA family, and HLA xx-like molecules (where "xx" denotes a particular HLA type), are synonyms. See, *e.g.*, the HLA-A2 motif and super motifs are detailed in **Table 4** ~~**Tables 4A, 4B, 4C, and 4D**~~.

Please replace paragraph [0054] with the following amended paragraph:

[0054] "Synthetic peptide" refers to a peptide that is **obtained** ~~**abtained**~~ from a non-natural source, *e.g.*, is man-made. Such peptides may be produced using such methods

as chemical synthesis or recombinant DNA technology. "Synthetic peptides" include "fusion proteins."

Please replace paragraph [0058] with the following amended paragraph:

[0058] The term "motif" refers to a pattern of residues in an amino acid sequence of defined length, preferably a peptide of less than about 15 amino acid residues in length, or less than about 13 amino acid residues in length, usually from about 8 to about 13 amino acid residues (*e.g.*, 8, 9, 10, 11, 12, or 13) for a class I HLA motif and from about 6 to about 25 amino acid residues (*e.g.*, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) for a class II HLA motif, which is recognized by a particular HLA molecule. Motifs are typically different for each HLA protein encoded by a given human HLA allele. These motifs often differ in their pattern of the primary and secondary anchor residues. In preferred embodiments, an MHC class I motif identifies a peptide of 9, 10, **or [[0r]]** 11 amino acid residues in length.

Please replace paragraph [0060] with the following amended paragraph:

[0060] A "primary anchor residue" is an amino acid at a specific position along a peptide sequence which is understood to provide a primary contact point between the immunogenic peptide and the HLA molecule. One, two or three, primary anchor residues within a peptide of defined length minimally defines a "motif" for an immunogenic peptide. These residues are understood to fit in close contact with peptide binding grooves of an HLA molecule, with their side chains buried in specific pockets of the binding grooves themselves. In one embodiment of an HLA class I motif, the

primary anchor residues are located at position 2 (from the amino terminal position) and at the carboxyl terminal position of a peptide epitope in accordance with the invention. The primary anchor positions for **various motifs and supermotifs** ~~**the A2 motif and supermotif**~~ of HLA Class I are set forth in Tables **3, 3a and 4** ~~**4A, 4B, 4C, and 4D**~~. For example, analog peptides can be created by altering the presence or absence of particular residues in these anchor positions. Such analogs are used to modulate the binding affinity of an epitope comprising a particular motif or supermotif.

Please replace paragraph [0061] with the following amended paragraph:

[0061] A "secondary anchor residue" is an amino acid at a position other than a primary anchor position in a peptide which may influence peptide binding. A secondary anchor residue occurs at a significantly higher frequency among HLA-bound peptides than would be expected by random distribution of amino acid residues at a given position. A secondary anchor residue can be identified as a residue which is present at a higher frequency among high or intermediate affinity binding peptides, or a residue otherwise associated with high or intermediate affinity binding. The secondary anchor residues are said to occur at "secondary anchor positions." For example, analog peptides can be created by altering the presence or absence of particular residues in these secondary anchor positions. Such analogs are used to finely modulate the binding affinity of an epitope comprising a particular motif or supermotif. The terminology "fixed peptide" is generally used to refer to an analog peptide that has changes in primary **anchor** ~~**anchore**~~ position; not secondary. A "cryptic epitope" elicits a response by immunization with an

isolated peptide, but the response is not cross-reactive *in vitro* when intact whole protein, which comprises the epitope, is used as an antigen.

Please replace paragraph [0068] with the following amended paragraph:

[0068] In the formulae representing selected specific embodiments of the present invention, the amino- and carboxyl-terminal groups, although not specifically shown, are in the form they would assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single letter or a capital first letter of a three-letter symbol, and the D-form for those amino acid residues having D-forms is represented by a lower case single letter or a lower case three letter symbol. However, when three letter symbols or full names are used without capitals, they may refer to L amino acid residues. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or "G". The amino acid sequences of peptides set forth herein are generally designated using the standard single letter symbol. (A, Alanine; C, Cysteine; D, Aspartic Acid; E, Glutamic Acid; F, Phenylalanine; G, Glycine; H, Histidine; I, Isoleucine; K, Lysine; L, Leucine; M, Methionine; N, Asparagine; P, Proline; Q, Glutamine; R, Arginine; S, Serine; T, Threonine; V, Valine; W, Tryptophan; and Y, Tyrosine.) In addition to these symbols, "B" in the single letter abbreviations used herein designates α-amino butyric acid. In some embodiments, α-amino butyric acid may be interchanged with cysteine.

Acronyms used herein are as follows:

APC:	Antigen presenting cell
CD3:	Pan T cell marker
CD4:	Helper T lymphocyte marker
CD8:	Cytotoxic T lymphocyte marker
CEA:	Carcinoembryonic antigen (see, *e.g.*, SEQ ID NO: 363)
CTL:	Cytotoxic T lymphocyte
DC:	Dendritic cells. DC function as potent antigen presenting cells by stimulating cytokine release from CTL lines that are specific for a model peptide derived from hepatitis B virus. *In vivo* experiments using DC pulsed *ex vivo* with an HBV peptide epitope have stimulated CTL immune responses *in vivo* following delivery to naïve mice.
DLT:	Dose-limiting toxicity, an adverse event related to therapy.
DMSO:	Dimethylsulfoxide
ELISA:	Enzyme-linked immunosorbant assay
E:T:	Effector:Target ratio
G-CSF:	Granulocyte colony-stimulating factor
GM-CSF:	Granulocyte-macrophage (monocyte)-colony stimulating factor
HBV:	Hepatitis B virus
HER2/neu:	A tumor associated antigen; c-erbB-2 is a synonym (see, *e.g.*, SEQ ID NO: **12** **[[364]]**)
HLA:	Human leukocyte antigen
HLA-DR:	Human leukocyte antigen class II
HPLC:	High Performance Liquid Chromatography
HTC:	Helper T Cell
HTL:	Helper T Lymphocyte. A synonym for HTC.
ID:	Identity
IFNγ:	Interferon gamma
IL-4:	Interleukin-4
IV:	Intravenous
$LU_{30\%}$:	Cytotoxic activity for 10^6 effector cells required to achieve 30% lysis of a target cell population, at a 100:1 (E:T) ratio.
MAb:	Monoclonal antibody
MAGE:	Melanoma antigen (see, *e.g.*, SEQ ID NO: **13** **[[365]]** and **14** **[[366]]** for MAGE2 and MAGE3, respectively)
MLR:	Mixed lymphocyte reaction
MNC:	Mononuclear cells
PB:	Peripheral blood
PBMC:	Peripheral blood mononuclear cell
ProGP™:	Progenipoietin™ product (Searle, St. Louis, MO), a chimeric flt3/G-CSF receptor agonist.
SC:	Subcutaneous
S.E.M.:	Standard error of the mean
QD:	Once a day dosing
TAA:	Tumor Associated Antigen
TNF:	Tumor necrosis factor

WBC: White blood cells

Please replace paragraph [0071] with the following amended paragraph:

[0071] The isolated epitopes and analogs of the invention are all class I binding peptides, *i.e.*, CTL peptides. In particular, the epitopes and analogs of the invention comprise an A2 motif or supermotif. Epitopes and analogs of the invention are those set forth in Table 1 **[[3]]** (SEQ ID NOs:1-10). A2 epitopes and analogs of the invention may be referred to herein as "epitopes" and "analogs" or referred to by Table or referred to by SEQ ID NO. Other epitopes and analogs are referred to herein as CTL epitopes or CTL peptides and HTL epitopes or HTL peptides.

Please replace paragraph [0072] with the following amended paragraph:

[0072] **Peptides and Polynucleotides**. In some embodiments, the invention is directed to an isolated peptide comprising or consisting of an epitope and/or analog, wherein the epitope or analog consists of a sequence selected from those in Table 1 **[[3]]** (SEQ ID NOs:1-10).

Please replace paragraph [0073] with the following amended paragraph:

[0073] Preferably, the peptide comprises or consists of an epitope or analog consisting of a sequence in Table 1 **[[3]]**.

Please replace paragraph [0085] with the following amended paragraph:

[0085] The peptide may also exclude any one or several epitopes and/or analogs selected from those in Table **1** **[[3]]** (SEQ ID NOs:1-10). Epitopes/analogs which may preferably be excluded from peptides of the invention are SEQ ID NOs:1-10.

Please replace paragraph [0095] with the following amended paragraph:

[0095] The HTL peptide may comprise a synthetic peptide such as a Pan-DR-binding epitope (*e.g.*, a PADRE® peptide, Epimmune Inc., San Diego, CA, described, for example, in U.S. Patent Number 5,736,142), for example, having the formula aKXVAAZTLKAAa, where "X" is either cyclohexylalanine, phenylalanine, or tyrosine; "Z" is either tryptophan, tyrosine, histidine or asparagine; and "a" is either D-alanine or L-alanine (SEQ ID NO: **42** **[[746]]**). Certain pan-DR binding epitopes comprise all "L" natural amino acid residues; these molecules can be provided as peptides or in the form of nucleic acids that encode the peptide. See also, U.S. Patent Nos. 5,679,640 and 6,413,935.

Please replace paragraph [00119] with the following amended paragraph:

[00119] Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, *e.g.*, the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), **α-factor** ~~**∀-factor**~~, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in

appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, *e.g.*, stabilization or simplified purification of expressed recombinant product.

Please replace paragraph [00131] with the following amended paragraph:

[00131] Known methods in the art can be used to enhance delivery and uptake of a polynucleotide *in vivo*. For example, the polynucleotide can be complexed to polyvinylpyrrolidone (PVP), to prolong the localized bioavailability of the polynucleotide, thereby enhancing uptake of the polynucleotide by the **organism** ~~**organisum**~~ (*see e.g.*, U.S. Patent No. 6,040,295; EP 0 465 529; WO 98/17814). This **approach** ~~**approache**~~ is thought to be more effective than inoculation with merely "naked" DNA. PVP is a polyamide that is known to form complexes with a wide variety of substances, and is chemically and physiologically inert.

Please replace paragraph [00180] with the following amended paragraph:

[00180] The corresponding family of HLA molecules (*i.e.*, the HLA-A2 supertype that binds these peptides) is comprised of at least: A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, and A*6901. Other allele-specific HLA molecules predicted to be members of the A2 superfamily are shown in ~~**Tables 4D**~~ **Tables 4** and 6. As explained in detail below, binding to each of the individual allele-specific HLA molecules can be modulated by substitutions at the

primary anchor and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Please replace paragraph [00182] with the following amended paragraph:

[00182] Thus, the HLA-A*0201 motif comprises peptide ligands with L, I, V, M, A, T, or Q as primary anchor residues at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope. For this motif-supermotif relationship the preferred and less preferred/tolerated residues that characterize the primary anchor positions of the HLA-A*0201 motif are identical to the residues describing the A2 supermotif. (For reviews of relevant data, *see, e.g.*, del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Ruppert *et al.*, *Cell* 74:929-937, 1993; Sidney *et al.*, *Immunol. Today* 17:261-266, 1996; Sette and Sidney, *Curr. Opin. in Immunol.* 10:478-482, 1998). Secondary anchor residues that characterize the A*0201 motif have additionally been defined (*see, e.g.*, Ruppert *et al.*, *Cell* 74:929-937, 1993). These secondary anchors are shown in **Table 4** ~~**Tables 4A, 4B, and 4C**~~. Peptide binding to HLA-A*0201 molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Please replace paragraph [00191] with the following amended paragraph:

[00191] In brief, the analoging strategy utilizes the motifs or supermotifs that correlate with binding to certain HLA molecules. Analog peptides can be created by substituting amino acid residues at primary anchor, secondary anchor, or at primary and secondary anchor positions. Generally, analogs are made for peptides that already bear a motif or

supermotif. As noted herein, preferred primary and secondary anchor residues of supermotifs and motifs for HLA-A2 class I binding peptides are shown in Tables **3, 3a and 4** ~~4A, 4B, and 4C~~. For a number of the motifs or supermotifs in accordance with the invention, residues are defined which are deleterious to binding to allele-specific HLA molecules or members of HLA supertypes that bind the respective motif or supermotif (Table **4** **[[4C]]**). Accordingly, removal of such residues that are detrimental to binding can be performed in accordance with the present invention.

Please replace paragraph [00230] with the following amended paragraph:

[00230] Preferably, the following principles are utilized when selecting epitope(s) and/or analogs for inclusion in a vaccine, either peptide-based or nucleic acid-based formulations. Exemplary epitopes and analogs that may be utilized in a vaccine to treat or prevent TAA-associated disease are set out in Table **14** **[[3]]**. Each of the following principles can be balanced in order to make the selection. When multiple epitopes are to be used in a vaccine, the epitopes may be, but need not be, contiguous in sequence in the native antigen from which the epitopes are derived. Such multiple epitotes can refer to the order of epitopes within a peptide, or to the selection of epitopes that come from the same reagion, for use in either individual peptides or in a multi-epitopic peptide.

1.) Epitopes and/or analogs are selected which, upon administration, mimic immune responses that have been observed to be correlated with prevention or clearance of TAA-expressing tumors. For HLA Class I, this generally includes 3-4 epitopes and/or analogs from at least one TAA.

2.) Epitopes and/or analogs are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC_{50} of 500 nM or less, or for Class II an IC_{50} of 1000 nM or less. For HLA Class I it is presently preferred to select a peptide having an IC_{50} of 200 nM or less, as this is believed to better correlate not only to induction of an immune response, but to *in vitro* tumor cell killing as well. For HLA A1 and A24, it is especially preferred to select a peptide having an IC_{50} of 100 nM or less.

3.) Supermotif bearing-epitopes and/or analogs, or a sufficient array of allele-specific motif-bearing epitopes and/or analogs, are selected to give broad population coverage. In general, it is preferable to have at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess the breadth of population coverage.

4.) For cancer-related antigens, it can be preferable to select analogs instead of or in addition to epitopes, because the patient may have developed tolerance to the native epitope.

5.) Of particular relevance are "nested epitopes." Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. For example, a nested epitope can be a fragment of an antigen from a region that contains multiple epitopes that are overleapping, or one epitope that is completely encompassed by another, *e.g.*, A2 peptides MAGE3.159 and MAGE3.160 are nested. A peptide comprising "transcendent nested epitopes" is a peptide that has both HLA class I and HLA class II epitopes in it. When providing nested epitopes, it is preferable to provide a sequence that has the greatest number of epitopes per provided sequence. Preferably, one avoids providing a

peptide that is any longer than the amino terminus of the amino terminal epitope and the carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a sequence comprising nested epitopes, it is important to evaluate the sequence in order to insure that it does not have pathological or other deleterious biological properties; this is particularly relevant for vaccines directed to infectious organisms.

6.) If a protein with multiple epitopes or a polynucleotide (*e.g.*, minigene) is created, an objective is to generate the smallest peptide that encompasses the epitopes of interest. This principle is similar, if not the same as that employed when selecting a peptide comprising nested epitopes. However, with an artificial peptide comprising multipe epitopes, the size minimization objective is balanced against the need to integrate any spacer sequences between epitopes in the polyepitopic protein. Spacer amino acid residues can be introduced to avoid junctional epitopes (an epitope recognized by the immune system, not present in the target antigen, and only created by the man-made juxtaposition of epitopes), or to facilitate cleavage between epitopes and thereby enhance epitope presentation. Junctional epitopes are generally to be avoided because the recipient may generate an immune response to that non-native epitope. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.

Please replace paragraph [00277] with the following amended paragraph:

[00277] Table 7 **[[5]]** below delineates the tumor antigen expression in breast, colon and lung. By targeting four TAA, the likelihood of the mutation of tumor cells (tumor

escape) into cells which do not express any of the tumor antigens is decreased. Preferably, the inclusion of two or more epitopes from each TAA serves to increase the likelihood that individuals of different ethnicity will respond to the vaccine and provides broadened population coverage.

Please replace paragraph [00279] with the following amended paragraph:

[00279] Table **8** **[[6]]** shows the incidence, 5-year survival rates, and the estimated number of deaths per year for these tumors in the U.S for each type of cancer in Table 5. In terms of estimated new cases, estimated deaths and 5 year survival rates each of these tumor types has a large unmet need. Globally, the incidence of these tumors is significantly greater.

Please replace paragraph [00280] with the following amended paragraph:

[00280] There is increasing evidence that HTL activity is critical for the induction of long lasting CTL responses (Livingston *et al. J. Immunol* 162:3088-3095 (1999); Walter *et al., New Engl. J. Med.* 333:1038-1044 (1995); Hu *et al., J. Exp. Med.* 177:1681-1690 (1993)). Therefore, one or more peptides that bind to HLA class II molecules and stimulate HTLs can be used in accordance with the invention. Accordingly, a preferred embodiment of a vaccine includes a molecule from the PADRE® family of universal T helper cell epitopes (HTL) that target most DR molecules in a manner designed to stimulate helper T cells. For instance, a pan-DR-binding epitope peptide having the formula: aKXVAAZTLKAAa, where "X" is either cyclohexylalanine, phenylalanine, or tyrosine; "Z" is either tryptophan, tyrosine, histidine or asparagine; and "a" is either

D-alanine or L-alanine (SEQ ID NO:42 **[[29]]**), has been found to bind to most HLA-DR alleles, and to stimulate the response of T helper lymphocytes from most individuals, regardless of their HLA type.

Please replace paragraph [00281] with the following amended paragraph:

[00281] A particularly preferred PADRE® molecule is a synthetic peptide, aKXVAAWTLKAAa (a = D-alanine, X = cyclohexylalanine) **(SEQ ID NO:1)**, containing non-natural amino acid residues, specifically engineered to maximize both HLA-DR binding capacity and induction of T cell immune responses.

Please replace paragraph [00282] with the following amended paragraph:

[00282] Alternative preferred PADRE® molecules are the peptides, aKFVAAWTLKAAa **(SEQ ID NO:19)**, aKYVAAWTLKAAa **(SEQ ID NO:20)**, aKFVAAYTLKAAa **(SEQ ID NO:21)**, aKXVAAYTLKAAa **(SEQ ID NO:22)**, aKYVAAYTLKAAa **(SEQ ID NO:23)**, aKFVAAHTLKAAa **(SEQ ID NO:24)**, aKXVAAHTLKAAa **(SEQ ID NO:25)**, aKYVAAHTLKAAa **(SEQ ID NO:26)**, aKFVAANTLKAAa **(SEQ ID NO:27)**, aKXVAANTLKAAa **(SEQ ID NO:28)**, aKYVAANTLKAAa **(SEQ ID NO:29)**, AKXVAAWTLKAAA (SEQ ID NO:30), AKFVAAWTLKAAA (SEQ ID NO:31), AKYVAAWTLKAAA (SEQ ID NO:32), AKFVAAYTLKAAA (SEQ ID NO:33), AKXVAAYTLKAAA (SEQ ID NO:34), AKYVAAYTLKAAA (SEQ ID NO:35), AKFVAAHTLKAAA (SEQ ID NO:36), AKXVAAHTLKAAA (SEQ ID NO:37), AKYVAAHTLKAAA (SEQ ID NO:38),

AKFVAANTLKAAA (SEQ ID NO:39), AKXVAANTLKAAA (SEQ ID NO:40), AKYVAANTLKAAA (SEQ ID NO:41) (a = D-alanine, X = cyclohexylalanine).

Please replace paragraph [00283] with the following amended paragraph:

[00283] In a presently preferred embodiment, the PADRE® peptide is amidated. For example, a particularly preferred amidated embodiment of a PADRE® molecule is conventionally written aKXVAAWTLKAAa-NH_2 **(SEQ ID NO:43)**.

Please replace paragraph [00284] with the following amended paragraph:

[00284] Competitive inhibition assays with purified HLA-DR molecules demonstrated that the PADRE® molecule aKXVAAWTLKAAa-NH_2 **(SEQ ID NO:43)** binds with high or intermediate affinity (IC_{50} ≤1,000 nM) to 15 out of 16 of the most prevalent HLA-DR molecules ((Kawashima *et al., Human Immunology* 59:1-14 (1998); Alexander *et al., Immunity* 1:751-761 (1994)). A comparison of the DR binding capacity of PADRE® and tetanus toxoid (TT) peptide 830-843, a "universal" epitope has been published (Panina-Bordignon *et al., Eur. J. Immunology* 19:2237-2242 (1989)). The TT 830-843 peptide bound to only seven of 16 DR molecules tested, while PADRE® bound 15 of 16. At least 1 of the 15 DR molecules that bind PADRE® is predicted to be present in >95% of all humans. Therefore, this PADRE® molecule is anticipated to induce an HTL response in virtually all patients, despite the extensive polymorphism of HLA-DR molecules in the human population.

Please replace paragraph [00285] with the following amended paragraph:

[00285] Early data from a phase I/II investigator-sponsored trial, conducted at the University of Leiden (C.J.M. Melief), support the principle that the PADRE® molecule aKXVAAWTLKAAa **(SEQ ID NO:1)**, possibly the amidated aKXVAAWTLKAAa - NH_2 **(SEQ ID NO:43)**, is highly immunogenic in humans (Ressing *et al.*, J. Immunother. 23(2):255-66 (2000)). In this trial, a PADRE® molecule was co-emulsified with various human papilloma virus (HPV)-derived CTL epitopes and was injected into patients with recurrent or residual cervical carcinoma. However, because of the late stage of carcinoma with the study patients, it was expected that these patients were immunocompromised. The patients' immunocompromised status was demonstrated by their low frequency of influenza virus-specific CTL, reduced levels of CD3 expression, and low incidence of proliferative recall responses after *in vitro* stimulation with conventional antigens. Thus, no efficacy was anticipated in the University of Leiden trial, rather the goal of that trial was essentially to evaluate safety. Safety was, in fact, demonstrated.

Please replace paragraph [00291] with the following amended paragraph:

[00291] The ability of ProGP generated DC to stimulate a CTL cell line was demonstrated *in vitro* using a viral-derived epitope and a corresponding epitope responsive CTL cell line. Transgenic mice expressing human HLA-A2.1 were treated with ProGP. Splenic DC isolated from these mice were pulsed with a peptide epitope derived from hepatitis B virus (HBV Pol 455) and then incubated with a CTL cell line that responds to the HBV Pol 455 epitope/HLA-A2.1 complex by producing IFNγ. The capacity of ProGP-derived splenic DC to present the HBV Pol 455 epitope was greater

than that of two positive control populations: GM-CSF and IL-4 expanded DC cultures, or purified splenic B cells (Figure **1B [[3B]]**). The left shift in the response curve for ProGP-derived spleen cells versus the other antigen presenting cells reveal that these ProGP-derived cells require less epitope to stimulate maximal IFNγ release by the responder cell line.

Please replace paragraph [00292] with the following amended paragraph:

[00292] The ability of *ex vivo* peptide-pulsed DC to stimulate CTL responses *in vivo* was also evaluated using the HLA-A2.1 transgenic mouse model. DC derived from ProGP-treated animals or control DC derived from bone marrow cells after expansion with GM-CSF and IL-4 were pulsed *ex vivo* with the HBV Pol 455 CTL epitope, washed and injected (IV) into such mice. At seven days post immunization, spleens were removed and splenocytes containing DC and CTL were restimulated twice *in vitro* in the presence of the HBV Pol 455 peptide. The CTL activity of three independent cultures of restimulated spleen cell cultures was assessed by measuring the ability of the CTL to lyse ^{51}Cr-labeled target cells pulsed with or without peptide. Vigorous CTL responses were generated in animals immunized with the epitope-pulsed ProGP derived DC as well as epitope-pulsed GM-CSF/IL-4 DC (Figure **1 [[4]]**). In contrast, animals that were immunized with mock-pulsed ProGP-generated DC (no peptide) exhibited no evidence of CTL induction. These data confirm that DC derived from ProGP treated mice can be pulsed *ex vivo* with epitope and used to induce specific CTL responses *in vivo*. Thus, these data support the principle that ProGP-derived DC promote CTL responses in a model that manifests human MHC Class I molecules.

Please replace paragraph [00295] with the following amended paragraph:

[00295] Vaccination is performed by injection or intravenous infusion of thawed cell product after the hematologic effects of ProGP in the patient have dissipated (*i.e.*, the hemogram has returned to baseline). Figure **2** **[[5]]** provides a flow chart of *ex vivo* pulsing of DC with peptides, washing of DC, DC testing, and cryopreservation. A more detailed description of the process is provided in the following Examples.

Please replace paragraph [00312] with the following amended paragraph:

[00312] A computer-based motif and algorithm search was performed on the primary amino acid sequences of CEA, MAGE-2/3, HER-2/neu, and p53 to predict peptides most likely to bind the MHC molecules of the five alleles of the HLA-A2 supertype family (Tables **4** **[[4D]]** and 6) (Sette, A. and J. Sidney, *Immunogenetics 50*:201-212 (1999)). Motif and algorithm-positive peptides were then synthesized and tested for binding to purified HLA-A2.1, the molecule most frequently expressed in humans as well as other MHC molecules of the HLA-A2 supertype family.

Please replace paragraph [00317] with the following amended paragraph:

[00317] Using the epitope selection process described above, nine epitopes were selected for the vaccine product. These epitopes, shown in **Table 1** ~~**Tables 3 and 7**~~, were chosen on the basis of demonstrating 1) broad tumor antigen coverage with a mix of CTL native sequence epitopes, fixed-anchor analogs and heteroclitic analogs; 2) high cross-reactive binding affinity for HLA-A2 supertype alleles; 3) immunogenicity in the *in vitro* human

primary CTL induction assay, particularly in generating CTL that respond to wild-type, epitope-expressing tumor cells; and, 4) wherever available, published reports in the literature showing primary or post-vaccination CTL responses in normal subjects or cancer patients.

Please replace paragraph [00319] with the following amended paragraph:

[00319] Four of the epitopes selected are fixed-anchor analogs that were modified for improved MHC binding. One fixed-anchor analog was derived from the well-characterized HER-2/neu.369 epitope, which has been shown to induce strong recall and post-vaccination CTL responses in cancer patients (Zaks, T. Z. and Rosenberg, S. A., *Cancer Res. 58*:4902-4908 (1998); Knutson, K. L., *et al., J Clin. Invest 107*:477-484 (2001)). By increasing supertype binding through substitution of both MHC anchor residues, the V2V9 analog of HER-2/neu.369 is expected to demonstrate even broader immunogenicity in HLA-A2 supertype individuals. The remaining fixed-anchor analogs (CEA.24V9, p53.139L2B3, and p53.149M2) were designed from epitopes identified in the selection process and they have not been tested previously in the clinic. The p53.139L2B3 analog contains an additional α-aminoisobutyric acid substitution at position 3 (a non-anchor position) to circumvent potential stability issues with the cysteine residue found in the wild-type epitope. As with the epitopes derived from wild-type sequences, all of the fixed-anchor analogs induce CTL that cross-react with the wild-type epitope presented by tumor cell lines ~~(Table 7)~~ (Keogh, E., *et al., J Immunol. 167*:787-796 (2001)).

Please replace paragraph [00326] with the following amended paragraph:

[00326] For the *in vivo* immunogenicity studies in HLA-A2.1/K^b transgenic mice, EP-2101 (prepared by an emulsification protocol (*see* Example 17) similar to that proposed for drug manufacture) was injected into mice and CTL responses against all of the epitopes in the vaccine were measured and compared to CTL responses induced by co-immunizing mice with each CTL epitope alone plus the PADRE® epitope, in Montanide® ISA 51 adjuvant. CTL responses were determined by measuring IFN-γ production by CTLs using an *in situ* ELISA (McKinney, D. M., et al., *J Immunol. Methods* 237:105-117 (2000)), following *in vitro* stimulation of splenocytes from immunized animals with peptide. As shown in Figure **3B [[6]]**, based on data gathered thus far from 6-10 independent experiments, the EP-2101 vaccine appears to demonstrate immunogenicity for a majority of CTL epitopes. For half of the CTL epitopes in EP-2101, strong CTL responses were observed that exceeded 50 secretory units (SU) of IFN-γ production (CEA.24V9, CEA.691H5, HER-2/neu.369V2V9, MAGE-2.157, and MAGE-3.112I5). The remaining epitopes demonstrated moderate to weak CTL responses (<10-50 SU), and these responses were generally associated with larger experimental variations as indicated by the larger standard deviation bars. As discussed above, these variations reflect the limitations of the transgenic mouse assay. However, despite the inherent variability of the assay, the multi-epitope CTL responses induced by the pool of CTL epitopes in EP-2101 appeared comparable to CTL responses induced in mice by each CTL epitope alone, when co-immunized with the PADRE® epitope in Montanide® ISA 51 adjuvant. Thus, overall, these experiments indicate that EP-2101 is immunogenic in HLA-A2.1/K^b transgenic mice. Additional experiments also indicate that EP-2101 can

induce HTL responses against the PADRE® epitope in HLA-A2.1/K^b transgenic mice, which are restricted by the mouse H-2 I-A^b allele (Alexander, J., *et al., Immunity. 1*:751-761 (1994)).

Please replace paragraph [00344] with the following amended paragraph:

[00344] The amino acid sequence of each peptide is given in **Table 1** ~~**Tables 3 and 7**~~.

Please replace paragraph [00352] with the following amended paragraph:

[00352] Solubility studies have been performed on the peptides, with solubility defined as a clear solution with no visible particulates (Table **15** **[[8]]**). Only 6 out of 10 peptides (1013.08, 1243.08, 1295.03, 1323.06, 1350.01 and 1352.03) were soluble at physiological pH. Therefore, the peptides were tested at 2-5 mg/ml in various aqueous acidic solutions and aqueous basic solutions, in addition to dimethylsulfoxide (DMSO).

Please replace paragraph [00353] with the following amended paragraph:

[00353] Table **16** **[[9]]** describes the specifications for the Drug Substance.

Please replace paragraph [00354] with the following amended paragraph:

[00354] The components and quantitative composition of EP-2101 are described in Table **17** **[[10]]**.

Please replace paragraph [00355] with the following amended paragraph:

[00355] The components used in the manufacture of the drug product are listed in Table **18 [[11]]**.

Please replace paragraph [00356] with the following amended paragraph:

[00356] The bulk drug product is prepared as shown in Figure 5. The bulk drug product is formulated into three solutions (*see* Tables **15 [[8]]** and **19 [[12]]**) based on the solubility of individual peptides in each of the three solvents and the solubility of the peptides when pooled. Briefly, to allow for aseptic processing, the 10 peptides are dissolved into either an acidic solution (0.1875 M acetic acid), a basic solution (0.1 M sodium hydroxide) or the organic solvent DMSO. These three peptide-containing pools are sterilized by filtration. Under aseptic conditions, these three peptide pools are combined, buffered, pH adjusted and then homogenized with Montanide® ISA 51 adjuvant under temperature-controlled conditions to form the drug product. The drug product, a stable 1:1 (w:w) emulsion, is then filled into 2 ml glass vials and stored at 2-8°C.

Please replace paragraph [00357] with the following amended paragraph:

[00357] Tables **20 [[13]]** and **21 [[14]]** describe the specifications for EP-2101 bulk drug product and drug product, respectively.

Please replace paragraph [00358] with the following amended paragraph:

[00358] Concentration of all peptide components in the EP-2101 Drug Product is assayed by RP-HPLC (conditions illustrated in Table **22 [[15]]**). A specified quantity of

the emulsion is mixed with a solution of 0.1% TFA in DMSO to form a two-layered mixture. Attempts to produce a clear, homogeneous solution for HPLC analysis by using solvents other than DMSO were unsuccessful. Sampling of the aforementioned two-phase mixture takes place by inserting a syringe or pipette through the top mineral oil layer and into the bottom, DMSO layer. The only sample taken for HPLC analysis is taken from the DMSO layer, in which all peptides are soluble. HPLC chromatography affords a distinct chromatographic peak for each peptide, which upon integration and comparison to a calibration curve yields the individual peptide concentration in the EP-2101 Drug Product. The biphasic nature of the sample introduces variability in the estimation of the full sample volume as well as the transfer of the sample. Subsequently, because of the complexity of sample preparation and handling, unusually large errors in determining the peptide concentration (as high as 30%) were observed. The large variability was not accompanied by major degradation product formation or other unusual physical changes of the sample, and is inferred to be a result of sample preparation and handling. For this reason the specification of ±50% of the intended concentration was set as a release and stability criterion. Attempts to improve the sample handling and, simplify the biphasic mineral oil-DMSO mixture are currently underway with a primary aim of narrowing the release and stability specifications.

Please replace paragraph [00360] with the following amended paragraph:

[00360] Development of a relevant potency assay is challenging because the EP-2101 vaccine is designed to specifically stimulate HLA-A2.1-restricted CTL responses in humans and not other species. One way to address this challenge is to measure the *in*

vivo potency of EP-2101 using mice that express the HLA-A2.1 molecule as a transgene (*i.e.* HLA-A2.1/K^b transgenic mice). The proposed EP-2101 potency assay is similar to the preclinical assay used to measure the immunogenicity of EP-2101 CTL epitopes in HLA-A2.1/K^b transgenic mice (*see* Example 13). It should be pointed out that the HLA-A2.1/K^b transgenic mouse assay has limitations in quantifying CTL responses, specifically: 1) only about 80% of the HLA-A2.1-restricted epitopes that are immunogenic in humans also induce CTL responses in transgenic mice (Wentworth, P.A., *et al., Eur. J. Immunol. 26*:97-101 (1996)), therefore, CTL responses against some epitopes cannot be quantified using this system, and; 2) using a number of approaches, we have found that *in vivo* CTL responses generated in HLA transgenic mice, whether induced by vaccination or natural infection, are variable from experiment-to-experiment due to individual animal differences and to the *in vitro* manipulation of primed T cells required for this assay method ~~(*e.g. see* **Figure 7**)~~. Although the potency assay has limitations common to *in vivo* bioassays, it provides a measurement of overall potency of EP-2101. Accordingly, it serves as an appropriate complement to the highly sensitive and quantitative analytical assays described above.

Please replace paragraph [00383] with the following amended paragraph:

[00383] ELISPOT assays to measure CTL or HTL responses induced by EP-2101 were performed according to previously published protocols (Lewis JJ, *et al., Int J Cancer* 87:391 (1998)). Briefly, flat bottom 96-well nitrocellulose plates (IP, Millipore) were coated with IFN-γ mAb (10 μg/ ml, clone R4-6A2, PharMingen) and incubated overnight at 4°C. After washing with PBS, plates were blocked with RPMI-10 medium

for 1 h at 37°C. Four × 10^5 $CD8^+$ cells or $CD4^+$ cells (isolated with Miltenyi isolation system from EP-2101-immunized splenocytes) and 5 × 10^4 Jurkat-A2.1/K^b cells (for $CD8^+$ cells) or 10^5 γ-irradiated naïve spleen cells (for $CD4^+$ cells, treated with erythrocyte lysis buffer) were added to each well. Wells also received 10 μg/ml of CTL or HTL peptide to test for induction of responses against EP-2101 epitopes or an identical concentration of an irrelevant peptide. The irrelevant peptide for the CD8 ELISPOT assay was the HCV core.132 peptide (DLMGYIPLV **(SEQ ID NO:17)** ~~**(SEQ ID NO:__)**~~) and the HCV NS3.1253 peptide (GYKVLVLNPSVAATL **(SEQ ID NO:18)** ~~**(SEQ ID NO:__)**~~) for the CD4 ELISPOT assay. After incubation, the plates were washed thoroughly with PBS/ 0.05% Tween 20 and biotinylated IFN-γ mAb (2 μg/ml, clone XMG1.2, PharMingen) was added to each well and incubated for 2-4 h at 37°C. After washing 4 times with PBS/0.05% Tween 20, Vectastain ABC peroxidase (Vectastain Elite kit; Vector laboratories, Inc., Burlingame, California, USA) was added to the wells and plates were incubated for 1 h at room temperature. The plates were washed again 3 times with PBS/ 0.05% Tween 20 followed by 3 washes with PBS. One hundred μl of AEC solution (Sigma Chemical Co) was added to develop the spots. The reaction was stopped after 4-6 minutes under running tap water. The spots were counted by computer-assisted image analysis (Zeiss KS ELISPOT Reader, Jena, Germany). The net number of spots/10^6 $CD8^+$ cells or $CD4^+$ cells was calculated as (number of spots against relevant peptide) - (number of spots with irrelevant control peptide) × 2.5.

In the Tables:

The attached sheet includes changes to Table 1. This sheet replaces the original Table 1.

Attachment: Amended Table 1 showing changes

Table 1 Sequence of Peptides in Drug Substance

Peptide Identification	Sequence	SEQ ID NO:	Epitope	Type
965.10	aKXVAAWTLKAAa	1	PADRE®	Universal Helper T Cell Epitope
1013.08	RLLQETELV	2	HER-2/neu.689	Wild-type
1090.01	YLQLVFGIEV	3	MAGE2.157	Wild-type
1243.08	LLTFWNPPV	4	CEA.24V9	Fixed-Anchor Analog
1295.03	SMPPPGTRV	5	p53.149M2	Fixed-Anchor Analog
1323.06	KLBPVQLWV	6	p53.139L2B3	Fixed-Anchor Analog
1334.10	KVFGSLAFV	7	HER-2/neu.369V2V9	Fixed-Anchor Analog
1350.01	YLSGADLNL	8	CEA.605D6	Heteroclitic Analog
1352.02	IMIGHLVGV	9	CEA.691H5	Heteroclitic Analog
1352.03	KVAEIVHFL	10	MAGE-3.112I5	Heteroclitic Analog

a = d-alanine, B = α-aminoisobutyric acid, X = cyclohexylalanine

In the Sequence Listing:

Please insert the attached sequence listing at the end of the specification, after the drawings.

Attachment: Sequence Listing